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Methods for imaging renal tubule cells

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Functional studies of renal tubules by conventional techniques, such as isotopic tracer or electrophysiologic methods, are limited both by epithelial cellular heterogeneity and by technical considerations, such as paracellular ion flows and unstirred layer effects. Although many approaches have been devised to correct for these complications, it has been difficult to measure the transport properties of individual nephron cells. The functional characteristics of single cells in renal tubules can now be directly assessed by quantitative light microscopic techniques. This approach involves the measurement of the cell volume changes which occur when solution osmolality or ionic composition are altered. The judicious use of such experiments in combination with the application of specific transport inhibitors allows determination of cell membrane water and ion permeabilities as well as characterization of the ion transporters in the individual cell membranes which mediate transepithelial solute movements. The mechanisms by which renal tubule cells maintain their volume and ionic composition in the face of dramatically varying solute loads and transcellular transport rates may also be determined. Visualization of specific cell types in a heterogenous epithelium, such as the cortical collecting tubule, may make it feasible to conduct both optical and microelectrode studies on the same cell. Furthermore, the use of low light level video methods in conjunction with fluorescent probes should enable study of renal tubule cell biological processes such as receptor–ligand interaction, insertion and retreival of membrane transport proteins, and intracellular events such as organelle movement, endo- and exocytosis, and turnover of intracellular constituents.

Isolated renal tubules are well suited for light microscopic study. The absence of connective tissue or other supporting structures enables acquisition of high contrast images of the tubule cells. The small diameter (typically 30 μm) of the tubules eliminates unstirred layers within the lumen of the tubule. Careful design of the bath chamber permits the virtual elimination of unstirred layers on the basolateral cell surface. Renal tubules offer the opportunity to visualize epithelial cells both in the conventional *en face* manner as well as in lateral cross section. This article will review the principles and techniques for quantitative light microscopy of isolated renal tubular epithelial cells.

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Requirements for quantitation of renal cell volume

The working distance of high numerical aperture microscope lenses limits the design of the tubule perfusion apparatus. The tubule must be positioned near the bottom of the chamber and the pipets must be modified accordingly. The bottom of the chamber should consist of a coverglass of the appropriate thickness and refractive index for the objective lens utilized. The tubule lumen and bath perfusion systems must not cause disturbances in the focus or position of the tubule during changes of the perfusate. Temperature, gas composition and pH of the bathing solutions must be maintained constant during the solution changes. All changes in composition of either luminal or bath solutions should be completed significantly more rapidly than the time constant of the physiological process under investigation. This generally means that "step" changes in bath composition must be achieved, and that unstirred layer–related delays must be minimized. Images must be recorded at a frequency sufficient to characterize the rates of volume change induced by the processes under investigation. Because of high surface area–to–volume ratio of most renal tubular epithelial cells, cell volume changes occur rapidly. Tubule images must be recorded at rates which approach or exceed the video framing rate (30 frames/second, US Standard). Finally, selection of microscope lenses should be based on optimizations of working distance and numerical aperture rather than on other criteria such as chromatic or spherical aberration.

Microscope features

The study of isolated renal tubules must be carried out on the stage of an inverted microscope. The perfusion apparatus should be attached to a movable stage so that the tubule and pipets can be positioned in the optical axis of the microscope. The microscope should have a broad, stable base and a large well supported stage. Remote control of microscope focus is essential. We utilize a step motor attached to the fine focus knob as previously described [1]. A schematic diagram of our optical system is shown in Figure 1. We visualize the tubule with differential interference contrast (DIC) optics. The output of a tungsten halogen lamp (50 watt) is passed through a heat filter (or hot mirror) to remove the infrared components, and an interference filter to produce near monochromatic illumination. The optimal wavelength for illumination depends on the specific absorbance characteristics of the tubule preparation under study. We generally examine a new tissue at wavelengths in the visible spectrum varying from 450 to 700 nm to determine the illumination wavelength for optimal contrast. Rabbit cortical

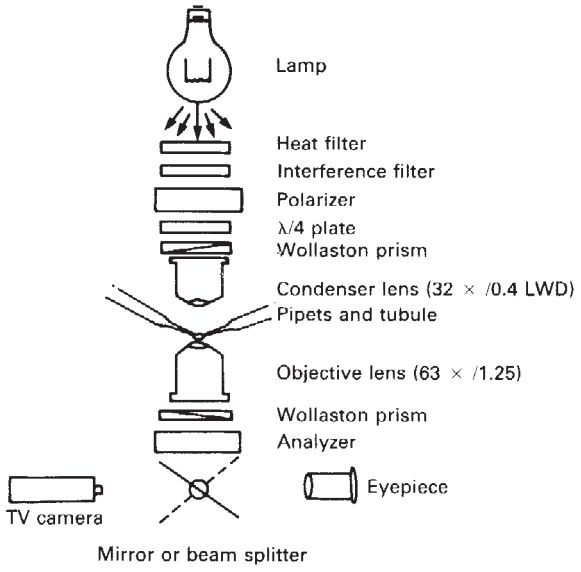

Fig. 1. *Schematic diagram of optics utilized for DIC microscopy of isolated renal tubules.*

collecting tubules are best visualized with a 620 nm interference filter (20 nm half width, Ditric Optics, Hudson, Massachusetts, USA). For DIC optics, the illumination beam must be linearly polarized by passing it through a polarizer, then circularly polarized by passage through a quarter wave plate. The merits of various systems of polarization have been discussed in detail previously [1]. The condenser lens must be of sufficient working distance to accommodate the perfusion pipets. The numerical aperture of the optical system will be limited by the condenser lens, and the system resolution will be diminished by the use of the long working distance condenser. We have chosen to utilize a dry long working distance objective lens (32×/0.4, E. Leitz, Inc., Rockleigh, New Jersey, USA) as our condenser. Other investigators have utilized a water immersion objective lens–condenser because of its higher numerical aperture (40×/0.75, Carl Zeiss, Inc., New York, New York, USA). We have rejected this approach because it prevents the rapid bath exchange required for most work on mammalian tubules. As shown in Figure 1, the illuminating beam must pass through a Wollaston prism positioned in the condenser mount or in the back focal plane of the objective lens condenser. We utilize a collar and Wollaston prism slider system (Zeiss) with our condenser–objective lens.

The objective lenses utilized in our experiments are a 100×/1.32 planapochromat (Leitz) and a 63×/1.25 Neofluor (Zeiss). Both of these lenses were selected because of their long working distance and high numerical aperture. Fully corrected lenses such as the planapochromat are not necessary because of the monochromatic nature of the illumination beam. Flatness of field at the edges is also not critical to quantitative microscopy of tubules because only the central 14 mm of the field is typically recorded by a video camera. The ideal lens for renal tubule microscopy then is the 63×/1.25 Neofluor because of its long working distance (500 μm).

A Wollaston prism is positioned in the back focal plane of the objective lens to recombine the beams. The analyzer in our system is a Glan–Taylor type calcite prism (Zeta Int. Corp., Mt. Prospect, Illinois, USA) in the nosepiece of our microscope (Diaphot, Nikon Inc., Garden City, New Jersey, USA) in the position normally occupied by the filter cube. Calcite prisms offer significant advantages over conventional film analyzers as previously described [1].

Improvements in image quality can be achieved by increasing the numerical aperture of the condenser lens. Efforts are underway to modify the geometry of standard condenser lenses (D. DiBona, personal communication) or to redesign the perfusion systems to permit the use of a high numerical aperture objective lens as a condenser (Spring and Strange, unpublished observations). Recent developments in confocal laser scan microscopy [2] may eventually lead to high resolution images of renal tubules without the constraints imposed by long working distance optics.

Perfusion apparatus and manipulation

Conventional tubule perfusion V-tracks can be utilized for quantitative microscopic studies of tubules. It is necessary to move the perfusion apparatus together with the perfusion chamber to position the preparation in the optical path. We attach the manipulators and perfusion apparatus directly to the microscope stage [3]. Our system employs optical translators (Klinger Scientific Corp., Richmond Hill, New York; or Daedal Inc., Harrison City, Pennsylvania, USA) to position the perfusion apparatus. These translators are preferable to the dovetail sliders typically supplied with the perfusion apparatus because of their superior stability, and movement accuracy. It is relatively simple to motorize such translators (Oriel Corp., Stratford, Connecticut, USA) to further reduce the likelihood of unwanted movements.

Bath chamber design

There were a number of crucial experimental requirements considered when designing a bath chamber for our recent studies on cell membrane water permeability in cortical collecting duct [3]. These requirements included: 1) minimal tubule focal displacements before, during and after serosal solution switches; 2) rapid changes in serosal solution composition; 3) minimization of serosal unstirred layer thickness; 4) maintenance of proper bath pH, gas composition and temperature.

Construction of our bath chamber is relatively simple (Figs. 2 and 3). A slot is machined into a plexiglass disc and the bottom of the slot is covered with a 25 mm diameter glass coverslip attached with silicone rubber cement. The slot is divided into two sections referred to as the set-up and perfusion baths (Fig. 2). Tubules are transferred after dissection to the set-up bath and cannulated with a conventional perfusion and holding pipet arrangement [4]. The tip of the perfusion holding pipet is then manipulated into the perfusion bath while the larger shoulder portion remains in the set-up bath (Fig. 3). This arrangement is crucial for rapid perfusion bath exchange time in that flow disturbances caused by the holding pipet are confined to the set-up bath.

The distal end of the tubule is held in place using a collection pipet with a 6 mm long tip (80 to 100 μm O.D., 30 to 50 μm I.D.). A tight fit of the tubules in the holding pipet and exposed tubule length of 400 to 500 μm is important for minimizing movements of the preparation. Once the tubule is properly

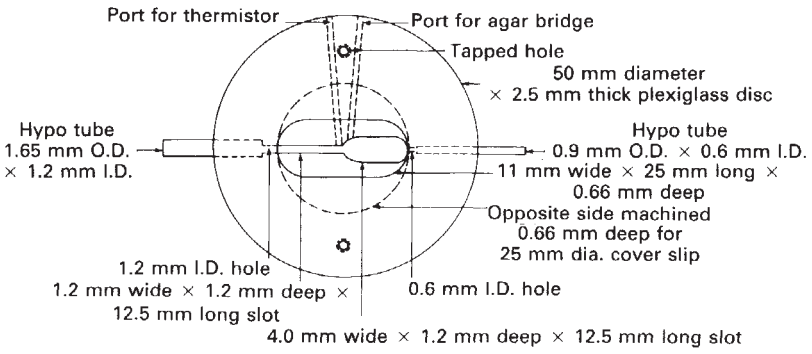

Fig. 2. *Diagram of the laminar flow perfusion bath showing the sites of fluid entry and exit as well as the ports for temperature probe and agar bridge.*

set–up, a small piece of coverglass is placed between the two pipets (Fig. 3). The bath solution adheres to the coverglass, producing a flat surface through which the condenser light can be properly focused onto the preparation.

Solutions enter the perfusion bath from a stainless steel, two–way manifold constructed from 1.2 mm I.D. tubing. Both the width and depth of the perfusion bath are also 1.2 mm. This critical design feature provides for laminar solution flow through the perfusion bath. Sudden expansion or contraction of fluid entering the bath chamber generates turbulent flow, slowing bath exchange time and causing tubule movements.

Bath perfusates are delivered to the chambers by gravity feed, and solution composition is switched using computer–controlled, solenoid–actuated pinch values (Model 370; Angar Scientific Controls, Cedar Knolls, New Jersey, USA). The total volume of the perfusion bath is about 80 μl. During experimental solution switches bath flow rate is 10 ml · min^{-1}. Even at flow rates twice this high we found that tubules remained extraordinarily stable before and during solution switches. Bath exchange times were monitored by focusing a 50 μm spot of light between a set of holding pipets, and monitoring the wash–in of Chicago Blue dye using a computer–interfaced photomultiplier tube (Tube Type 9826, 3/4″ assembly style A; Thorn EMI Gencom Inc., Plainview, New York, USA). Half–times for complete bath exchange at a flow rate of 10 ml · min^{-1} were between 55 to 60 msec (Fig. 2). Minimum half–times for collecting duct cell volume changes induced by serosal osmolality gradients were about 300 msec.

The laminar flow characteristics of the bath chamber reduced serosal unstirred layers to negligible values. The thickness of the unstirred layer was measured by observing the flow pattern of small latex beads (0.3 or 1.0 μm diameter) added to the serosal solution. At bath flow rates of 10 ml · min^{-1}, solution velocity was so high that the beads could only be observed as streaks on video disc images. These beads moved parallel to the tubule wall and flowed within 2 to 3 μm of the basement membrane. Since bead flow is confined solely to the bulk or convective solution flow, these results indicate that the serosal unstirred layer has a thickness $\leq$ 3 μm.

One of the most difficult technical problems encountered in these studies was proper control of bath temperature. The small chamber volume and extremely high flow rates produced large temperature gradients along the length of the perfusion bath. Solutions entering the manifold at 37°C typically reached the tubule at 30 to 31°C. When tubules were imaged at high power,

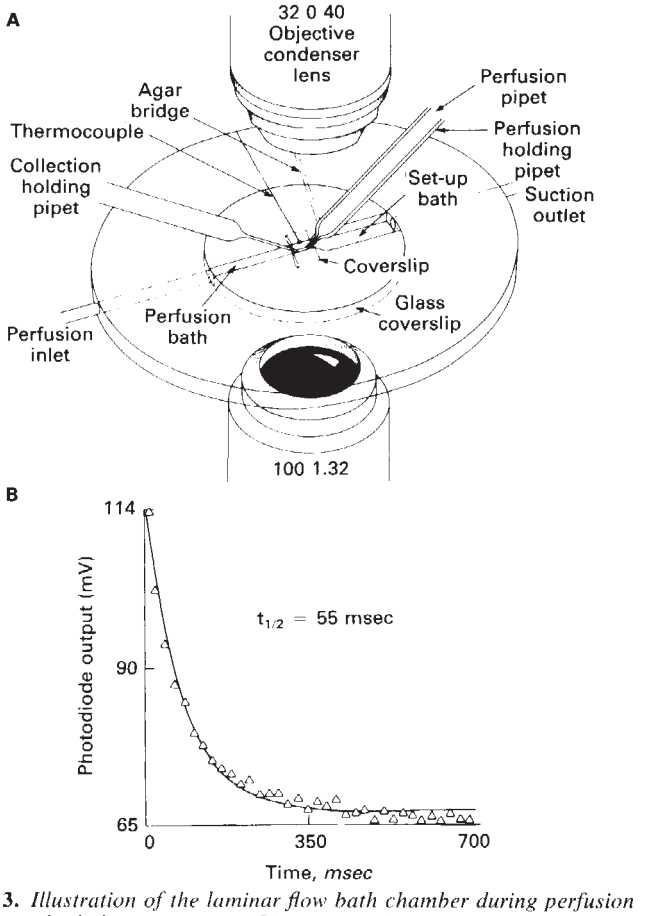

Fig. 3. *Illustration of the laminar flow bath chamber during perfusion of a renal tubule (top, part A).* Chamber washout curve measured with a photomultiplier tube in the region of the tip of the perfusion pipet (bottom, part B).

an additional heat loss (3 to 4°C) occurred through the oil immersion lens. This heat loss could not be compensated for by preheating solutions just before they entered the bath chamber. Raising the temperature of the solution by as little as 1°C above the gas equilibration temperature caused immediate degassing and bubble formation. Bubbles entering the perfusion bath at high flow rates either destroyed the tubule preparation or degraded image quality.

To solve this problem, we used water–jacketed perfusion

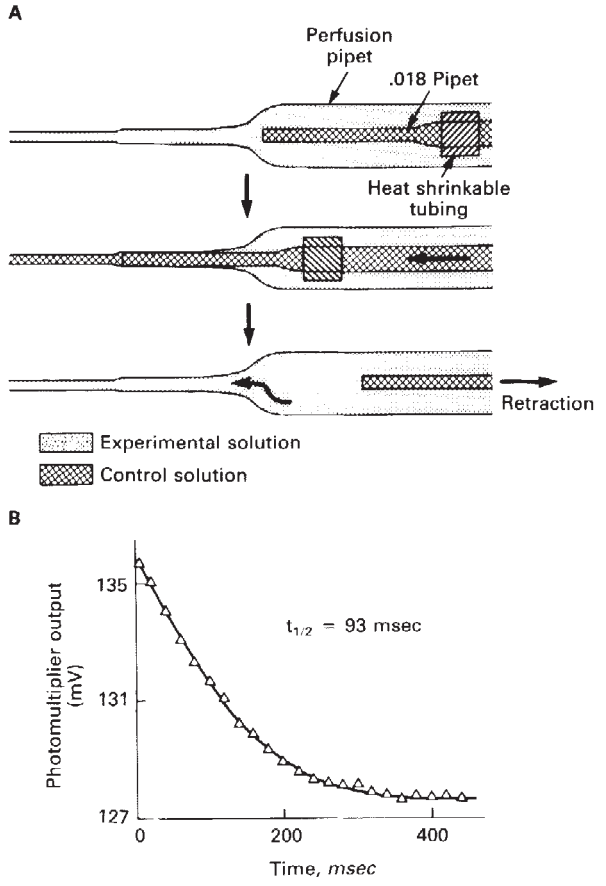

Fig. 4. *Diagram of the retractable pipet assembly utilized for rapid exchange of luminal perfusate (top, part A).* Dye washout curve in region of pipet tip after solution change by the retractable pipet method (bottom, part B).

reservoirs and perfusion lines. Perfusion reservoirs were maintained at 50 to 52°C and vigorously gassed with 7.5% CO_2 (balance air) to compensate for the decreased CO_2 solubility at higher temperatures. Perfusion lines were glass to prevent CO_2 loss and were water-jacketed with gasket-sealed, plexiglass tubing maintained at a temperature *no higher* than that in the perfusion reservoirs. When room temperature fell below 23°C we found that the bath temperature could not be maintained at 37°C. We could not compensate for the lowered room temperature by raising water jacket temperature, since $CaPO_4$ rapidly precipitated out of solution at temperatures above about 54°C. To alleviate this problem, we constructed a plexiglass environmental chamber around the microscope stage and nose piece which was maintained at 37°C. This prevented excessive heat loss through oil immersion objective lenses. Using this arrangement, bath temperature was maintained at 36 to 38°C and bath pH at 7.4. Temperature transients during solution switches were less than ± 0.3°C.

If finer temperature control is warranted, a small Peltier device (such as Model FCD 6-66-052; Melcor, Trenton, New Jersey, USA) controlled by a feedback amplifier can be placed between the stainless steel manifold and microscope stage to heat or cool solutions entering the chamber. Heating by the Peltier, however, most *never* exceed the temperature in the perfusion reservoirs or solution degassing will occur.

Perfusion pipet design

Rapid changes in luminal perfusate composition are crucial for accurate determination of the water permeability and electrophysiological properties of apical cell membranes and for studies of solute transport using cell volume measuring techniques. The development of the double-barrel perfusion pipet method for isolated nephrons [5] allows repeated changes in luminal solutions with millisecond half-times. Use of this pipet design, however, requires several trial switches of luminal perfusates to balance flow rates and pressures in the two pipet barrels. Typically, trial perfusate changes are followed by monitoring transepithelial potential during drug addition or ion substitutions. For our recent studies of cell membrane water permeability in rabbit cortical collecting tubules [3], we felt that trial perfusate switches were unacceptable, since they could directly affect apical water permeability by altering intracellular factors (such as, Ca^{+2}, pH), thought to control the insertion and retrieval of putative water channels into cell membranes of vasopressin-sensitive epithelia [6]. In addition, trial perfusate switches could alter cell volume and/or solute transport processes, which in turn could affect apical water movements and apparent water permeability.

To circumvent these problems, we developed a new pipet system which we refer to as the retractable pipet design (Fig. 4). Perfusion pipets were pulled in two stages with the first stage having an I.D. of 50 μm and a length of 500 μm. The inner (".018") pipet has a 1 mm long tip which is 50 μm in diameter. A small piece of heat-shrinkable teflon tubing is placed around the inner pipet to center it within the perfusion pipet. The inner pipet must make a fluid tight seal with the first stage of the perfusion pipet. When viewed under a dissecting microscope at high magnification, there should be no visible space between the walls of the two pipets for a distance of 200 to 300 μm.

Each combination of pipets is tested for leaks during set-up by drawing a small block of low viscosity (1 cs) silicone oil into the tip of the inner pipet, sealing the pipets together and then partially expelling the oil block. The perfusion pipet is then pressurized by a syringe. If the pipets are not properly sealed, a droplet of experimental solution flows out behind the oil and the pipets should be discarded.

Insertion of the inner pipet into the perfusion pipet is accomplished using a hydraulic microdrive arrangement (Fig. 5). The slave cylinder of the microdrive consists of an oil-filled 3 cc glass syringe, which is mounted directly to the pipet V-tracks. A piece of stainless steel tubing passes through the plunger of the syringe and out the tip through a gasket seal. The inner (".018") pipet is sealed into the stainless steel tubing using dental sticky wax (Kerr Sybron Corporation, Emeryville, California, USA). The two microdrive master cylinders consist of an oil-filled 12 cc plastic syringe and a 0.25 cc microburet syringe (Gilmont Instrument, Great Neck, New York, USA) separated by a three-way stopcock. The 12 cc syringe is used for coarse movements and rapid retraction of the inner pipet, while the microburet is used for sealing the two pipets tightly together.

Pipet exchange times were measured by focusing a 50 μm spot of light a short distance behind the tip of the perfusion

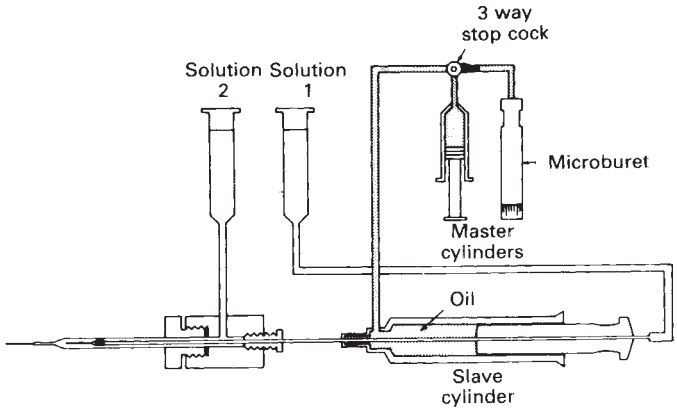

Fig. 5. *Schematic diagram of the hydraulic arrangement for the retractable pipet assembly shown in Figure 4.* Pressure on the large syringe or microburet causes the small slave syringe barrel to retract the central pipet.

pipet and monitoring the appearance of Chicago Blue dye in the pipet. We tested a variety of pipet combinations at a range of perfusion pressures and found that the half–times for solution changes were between 70 to 150 msec. Such exchange times were considerably more rapid than cell volume changes induced by alterations in luminal osmolality in rabbit cortical collecting duct [3]. The main disadvantage of our approach is that it can be used to make only a single solution switch in any given experiment. For electrophysiological studies where a number of cells from the same tubule are impaled and studied, the double–barrel pipet design is a more workable solution to the problem of rapid perfusate changes.

Cell morphology

The lack of large amounts of connective tissue and adherent interstitial tissue in most nephron segments allows acquisition of high contrast tubule cell images. In the rabbit cortical collecting duct we were able to clearly distinguish cell borders from basal to apical cell poles. We were also able to observe a wide variety of directed movements of intracellular organelles. The use of high resolution DIC microscopy in conjunction with fluorescence microscopy (Strange and Spring, unpublished observations) may allow detailed study of these movements, particularly as they relate to the control of cell function by hormones such as ADH [7] and environmental factors such as altered peritubular pCO_2 [8–10].

In rabbit cortical collecting ducts we were also able to clearly differentiate between principal and intercalated cell types. *En face* principal cell images were characterized as having a relatively low contrast, a hexagonal cell outline and the presence of a single cilium located on the apical cell membrane [3]. The function of the principal cell cilium is unknown at present, but the ability to visualize it in a living preparation may make possible its study in experiments where luminal perfusion has been stopped.

Recent physiological studies in rabbit [8, 9] and ultrastructural studies in rat [11] and rabbit [12] have suggested the presence of two distinct types of intercalated cells in cortical collecting ducts. We identified what appear to be at least three morphologically distinct populations of this cell type. One type

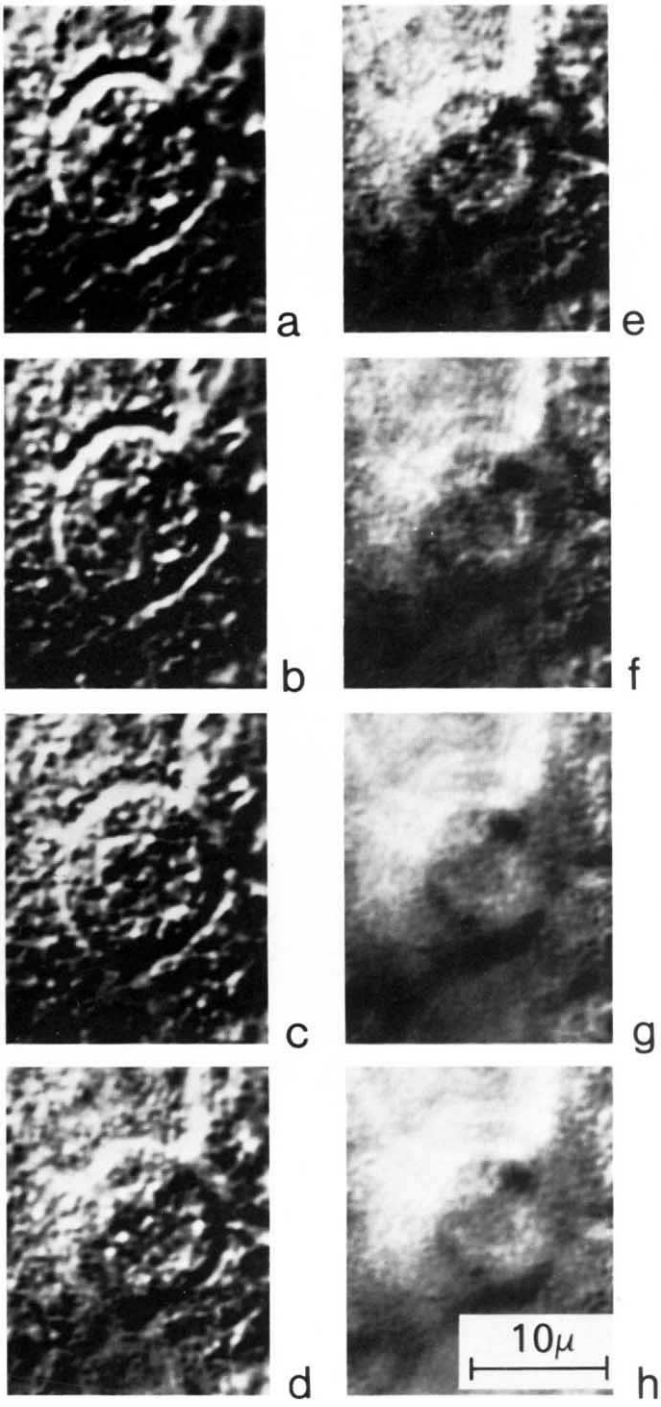

Fig. 6. *Composite diagram of optical sections of one type of intercalated cell visualized by DIC microscopy.* Sections are separated by 1.2 μm displacements of focus. Note that the cell has a rounded outline extending from basal (plate a) to apical pole (plate f).

has a circular cell outline which extends from the basal to apical pole of the cell. In any given preparation, these cells are taller than adjacent principal cells, causing them to bulge prominently into the tubule lumen (Fig. 6). A second intercalated cell type (Fig. 7) has a hexagonally–shaped basal membrane similar to that of the principal cell. Towards its apical pole, this cell type

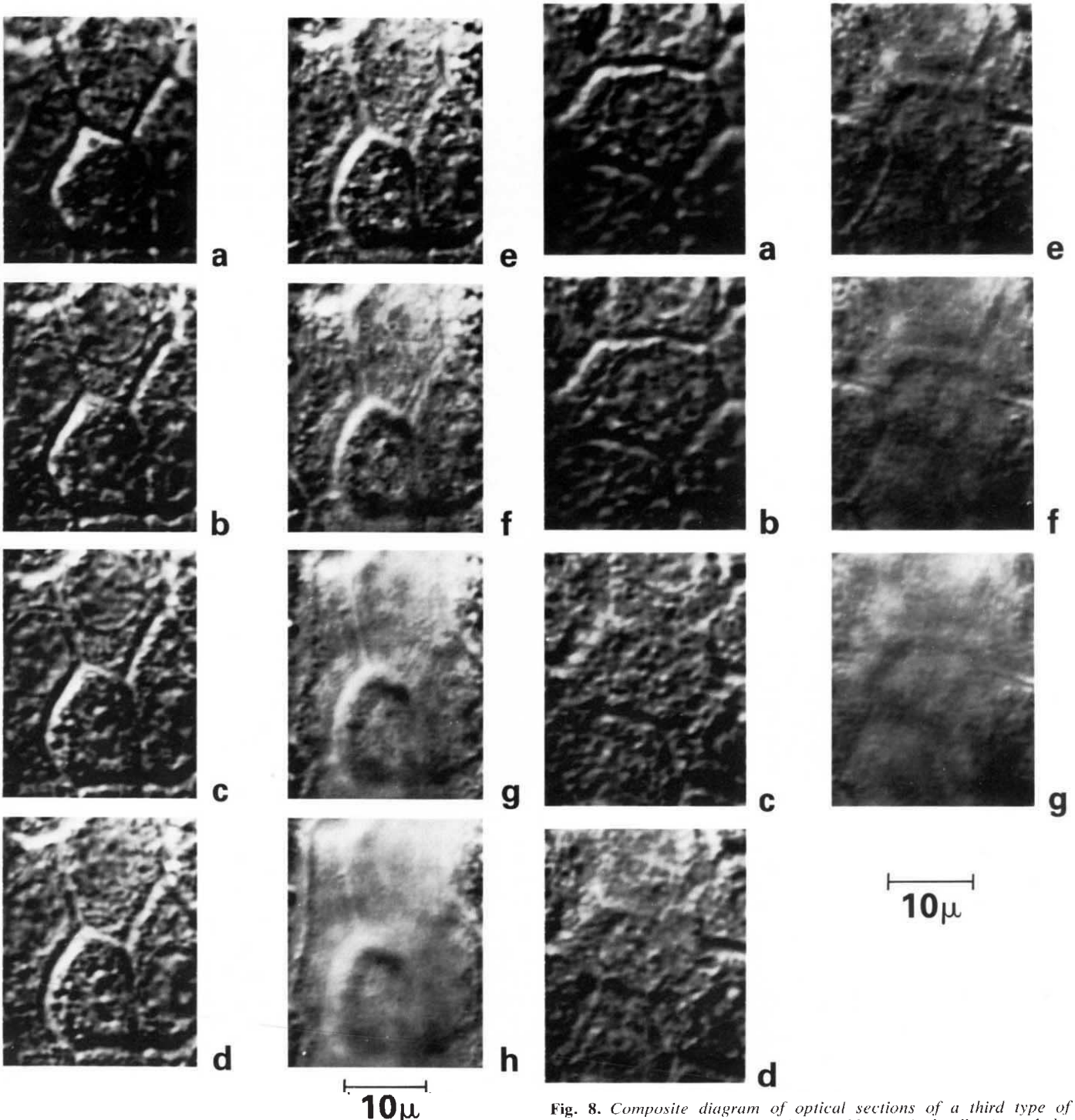

Fig. 7. *Composite diagram of optical sections of a second type of intercalated cell in rabbit cortical collecting tubule.* Sections 1.2 μm apart as in Figure 6. Note that this cell has a hexagonal outline extending from basal surface (plate a) to the middle of the cell (plate c), then gradually becomes rounded (plates e–g).

Fig. 8. *Composite diagram of optical sections of a third type of intercalated cell seen occasionally in rabbit cortical collecting tubule.* Sections 1.2 μm apart as in Figure 6. The hexagonal shape of the base of the cell (plates a,c) gradually takes on a circulat outline towards the cell apex (plates d–f). The small apical surface does not bulge into the lumen.

tapers and shows a characteristic circular cell outline. These cells have an apical surface area similar to the first intercalated cell type and also bulge out into the tubule lumen.

Infrequently we observed yet a third type of intercalated cell. These cells had a hexagonally–shaped basal membrane and tapered very rapidly at their apical pole, taking on the characteristic circular cell outline. The apical surface area exposed to the tubule lumen was extremely small in these cells and there

was clearly no apical bulging (Fig. 8). This morphology is very similar to the "flask–shaped" mitochondria–rich cells seen in toad [13] and turtle urinary bladder [14]. All three intercalated cell types also differed from principal cells in that they had a much higher relative contrast and contained numerous large intracellular structures which were possibly mitochondria.

Studies of collecting duct intercalated cell function have to date been largely indirect. Attempts to impale intercalated cells with microelectrodes have been unsuccessful primarily because of the inability to visualize this cell type in electrophysiological preparations. We are currently attempting to use our laminar flow bath chamber and optical arrangement in conjunction with microelectrodes to characterize intercalated cell function in detail. In addition, we are using cell volume measuring techniques to characterize solute entry and exit steps and cell volume regulatory mechanisms in this cell type.

The meaning of the morphological variations in intercalated cells are uncertain at present. Electron microscopy studies in rabbit and rat cortical collecting duct [11, 12] have described an intercalated cell similar to that shown in Figure 6 and a second cell type which is much more "flask–shaped" in appearance (Fig. 7). Tisher (personal communication) has termed the more circular cell a "Type A" intercalated cell and the "flask–shaped" cell a "Type B" intercalated cell. Studies by Schwartz and Al–Awqati [8, 9] have suggested that there are two functional types of intercalated cells in rabbit collecting duct. One of these secretes HCO_3 into the tubule lumen and the other secretes protons. Schwartz (personal communication) suggests that the "A" and "B" type cells secrete protons and HCO_3, respectively. We have also observed two functionally distinct populations of intercalated cells [3]. When cortical collecting tubules are exposed to a 390 mOsm serosal solution, one population of intercalated cells shrinks about 15% and remains shrunken for at least 40 min. A second group of intercalated cells also shrinks a similar amount but then shows almost complete regulatory volume increase behavior within 20 to 40 min. At this time we are not able to associate the ability to volume regulate with a particular intercalated cell type. Cell volume measurements are carried out on lateral tubule wall images where we have been unable to distinguish between the various types of intercalated cell.

The role played by the intercalated cell in salt and water homeostasis is clearly an important question in renal physiology. The combined use of optical and electrophysiological approaches should allow a detailed understanding of cellular transport processes, the role played by the intercalated cell in K^+ and acid–base adaptation, and the relationship between cell morphology and transport function.

Image analysis

The use of lens elements with high numerical apertures in conjunction with DIC optics yields images with a very shallow depth–of–field. The term depth–of–field refers to the ability to differentiate discrete points which are separated along the optical axis of the preparation. Advantage can be taken of this shallow depth–of–field to "optically section" a living cell and determine cell volume. In our studies of the cortical collecting tubule, cells were optically sectioned beginning at the basal cell membrane and proceeding towards the apical pole. At each 1.2 μm of focal displacement, a video image was recorded using a TV camera equipped with Newvicon tube (Model NC-655; Dage-MTI Inc., Michigan City, Indiana, USA) and a high resolution video disc recorder (Model VDR-1RA; Precision Echo, Mountain View, California, USA). Focal displacement is accomplished using a computer–interfaced stepping motor attached to the microscope fine focus drive. Great care should be taken in these types of studies to insure that the cell outline is flat and not contributing to the curvature of the tubule wall. The cross–sectional area of each optical section is determined at a later time using a computer to digitize the cell boundaries. Cell volume is determined using the cross–sectional areas and known focal displacement according to the method of Persson and Spring [15]. Because of the small cell volume and high surface area/volume ratios of most mammalian nephron segments, it is likely that many processes induced by experimental perturbations such as membrane potential changes, cell volume changes, and changes in intracellular ion concentrations will occur extremely rapidly. Technical approaches with adequate speed to record these processes are crucial for data interpretation. In our recent studies of water permeability in cortical collecting tubule [3], we found that cell volume changes induced by serosal osmolality gradients were complete in 600 to 700 msec. The time required to obtain one complete set (8 to 10 sections) of optical sections in a principal or intercalated cell is 1.5 to 2.0 seconds. Clearly, the optical sectioning technique could not be used to determine rates of cell volume changes under these circumstances. To acquire rate information then, we took advantage of the tubule geometry and imaged cross–sections of principal and intercalated cells in the lateral tubule wall. Once the proper focal plane is chosen, mucosal or serosal solution composition is switched and video images recorded at rates as high as 15 frames/second. Generally this is the maximum recording rate which can be obtained with video disc recorders due to limitations in the technology of the stepping motor which moves the recording head across the disc.

Higher recording speeds may be required to follow fast volume changes of some renal tubular cells. Collapsed proximal tubule segments were reported to respond so rapidly to osmotic challenges that initial rates were only obtainable in the 30 to 60 msec after the solution change [16]. Single field viewing of video taped images results in a 16 msec time resolution, but image quality is reduced. Higher speed images of volume changes can only be obtained with specially designed video cameras and recorders or by high speed photography. Alternatively photometric methods can be utilized which are not limited by video signals. Photodiodes or photomultiplier tubes positioned in the image plane have been widely used to measure capillary blood flow [17], membrane potential changes [18], and positional information [19]. These techniques can be combined with video methods to ensure stability of focus and image position [20, 21].

Measurement of cell volume changes

After the lateral wall images of renal tubules have been obtained, the cross–sectional area can be measured for determination of rates of cell volume changes. A critical assumption implicit in this approach is that a given cell type changes its volume in a symmetrical fashion. Imaging of a random cross–section of that particular cell type and determination of the rate of change of cross–sectional area in response to a given osmotic gradient should be representative of volume changes occurring

in the whole cell. We tested this assumption in the cortical collecting duct by measuring relative changes in cell volume and cell cross–sectional area for both principal and intercalated cells exposed to a 390 mOsm serosal solution [3]. Principal cell volume changed $-22.8 \pm 1.0\%$ ($N = 8$ tubules) whereas cell cross–sectional area changed $-22.6 \pm 0.8\%$ ($N = 9$), respectively. Similar results were obtained with intercalated cells [3]. These observations support the assumption that cell volume in cortical collecting ducts changes in a symmetrical fashion and that measurements of cross–sectional area changes can be used as measurements of the rate of cell volume change.

The cross–sectional area method is also a more sensitive measure of absolute volume changes. Small variations (about 2%) in cell cross–sectional area can be accurately quantified by imaging a random cell cross–section in the lateral tubule wall. Conversely, we estimated that in cortical collecting tubule, we could at best detect a minimum cell volume change of about 5 to 10% using the optical sectioning method.

Digital image processing

Digital processing of video images has resulted in dramatic improvements in contrast and resolution of cell organelles [22, 23]. The techniques employed involve substraction of out–of–focus background images to remove mottle and inhomogeneities in the microscopic field, adjustment of gain and offset to enhance contrast, edge enhancement to sharpen fine details, and false coloring to differentiate between subtle differences in the gray level of images [1,24]. Image processing equipment is expensive and some systems are very complex. A relevant question for renal physiologists at this time is whether it is worthwhile to invest in such approaches for the study of renal tubules. At present, our experience is that digital processing of renal tubule cell images does not lead to significant improvements in the images or to meaningful new insights into the physiology of these cells. One potentially valuable area of application of image processing is in the superposition of fluorescence and transmitted light images for the localization of fluorescent probes or structures. Efforts are underway in our laboratory and in several others [25, 26] to utilize image processing to obtain accurate measurements of intracellular pH or calcium in epithelial cells labelled with fluorescent probes.

Conclusions

The ability to measure cell volume and rates of cell volume change in isolated nephrons should provide new and important advances in understanding renal function. It is now possible for the first time to assign specific transport functions to different cell types in a single nephron segment (such as principal and intercalated cells). Studies of the mechanisms of ouabain–induced cell swelling can be used to elucidate major solute entry and exit steps in individual cells as has been done in *Necturus* gallbladder [27], *Amphiuma* diluting segment [28] and rabbit papillary epithelium [29]. The role played by hormones and acute and chronic physiological disturbances in controlling cell function can now be examined. Detailed studies of cell volume regulation can be carried out. These investigations are particularly important for understanding how nephron segments maintain cellular homeostasis in the face of dramatically varying solute loads and transcellular transport rates, and how cells in the renal medulla respond to changes in extracellular osmolality [30]. Finally, optical studies can be combined with electrophysiological measurements of intracellular ion activities to determine whether observed concentration changes are due to alterations in putative transport mechanisms or are merely due to changes in cell volume.

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